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L9: Entry 1 of 4

File: USPT

Apr 30, 2002

US-PAT-NO: 6379553

DOCUMENT-IDENTIFIER: US 6379553 B1

TITLE: Polymerase enhancing factor (PEF) extracts, PEF protein complexes, isolated PEF proteins, and methods for purifying and identifying same

DATE-ISSUED: April 30, 2002

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hogrefe; Holly	San Diego	CA		

US-CL-CURRENT: 210/656; 435/91.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw Desc
Image												

☐ 2. Document ID: US 6333165 B1

L9: Entry 2 of 4

File: USPT

Dec 25, 2001

US-PAT-NO: 6333165

DOCUMENT-IDENTIFIER: US 6333165 B1

TITLE: Methods for identifying polymerase enhancing factor (PEF)

DATE-ISSUED: December 25, 2001

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hogrefe; Holly	San Diego	CA		

US-CL-CURRENT: 435/7.4

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMC	Draw Desc
Image											

☐ 3. Document ID: US 6183997 B1

L9: Entry 3 of 4

File: USPT

Feb 6, 2001

US-PAT-NO: 6183997

DOCUMENT-IDENTIFIER: US 6183997 B1

TITLE: Polymerase enhancing factor (PEF) extracts PEF protein complexes isolated PEF proteins and methods for purifying and identifying same

DATE-ISSUED: February 6, 2001

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hogrefe; Holly	San Diego	CA		

US-CL-CURRENT: 435/91.2; 536/23.7, 536/24.1

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KIMC	Draw Desc
Image											

☐ 4. Document ID: US 6379553 B1, WO 9842860 A1, EP 1007718 A1, US 6183997 B1, US 6333165 B1, JP 2002505572 W

L9: Entry 4 of 4

File: DWPI

Apr 30, 2002

DERWENT-ACC-NO: 1998-542284

DERWENT-WEEK: 200235

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TITLE: Polymerase enhancing factor proteins, extracts and complexes - improve the polymerisation activity of nucleic acid polymerases, for use in amplification, sequencing and replication

INVENTOR: HANSEN, C J; HOGREFE, H

## PATENT-ASSIGNEE:

ASSIGNEE	CODE
STRATAGENE	STRAN

PRIORITY-DATA: 1997US-0957709 (October 24, 1997), 1997US-0822774 (March 21, 1997), 2000US-0632711 (August 4, 2000), 2000US-0632703 (August 4, 2000)

## PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 6379553 B1	April 30, 2002		000	C07K001/16
WO 9842860 A1	October 1, 1998	E	160	C12P019/34
EP 1007718 A1	June 14, 2000	E	000	C12P019/34
US 6183997 B1	February 6, 2001		000	C12P019/34
US 6333165 B1	December 25, 2001		000	G01N033/58
JP 2002505572 W	February 19, 2002		184	C12N015/09

DESIGNATED-STATES: CA JP US AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE AT CH DE FR GB IT LI NL

## APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
US 6379553B1	March 21, 1997	1997US-0822774	Div ex
US 6379553B1	August 4, 2000	2000US-0632703	
US 6379553B1		US 6183997	Div ex
WO 9842860A1	March 20, 1998	1998WO-US05497	
EP 1007718A1	March 20, 1998	1998EP-0911883	
EP 1007718A1	March 20, 1998	1998WO-US05497	
EP 1007718A1		WO 9842860	Based on
US 6183997B1	March 21, 1997	1997US-0822774	
US 6333165B1	March 21, 1997	1997US-0822774	Div ex
US 6333165B1	August 4, 2000	2000US-0632711	
US 6333165B1		US 6183997	Div ex
JP2002505572W	March 20, 1998	1998JP-0545828	
JP2002505572W	March 20, 1998	1998WO-US05497	
JP2002505572W		WO 9842860	Based on

INT-CL (IPC): C07 K 1/16; C07 K 14/195; C07 K 16/12; C12 N 9/12; C12 N 15/09; C12 P 19/34; C12 P 21/02; C12 Q 1/68; G01 N 33/58; G06 F 17/30; C12 N 15/09; C12 R 1/01

ABSTRACTED-PUB-NO: US 6183997B

BASIC-ABSTRACT:

A new non-naturally occurring composition (I) comprises at least 1 components with nucleic acid polymerase enhancing (PE) activity selected from: (a) an isolated or purified natural PE protein from a bacterial, eukaryotic or archael source; (b) a wholly or partially synthetic protein having the same sequence as the natural protein, or an analogue with PE activity; (c) PE mixts. of at least 1 natural, or wholly or partially synthetic proteins; (d) a PE protein complex of at least 1 natural or synthetic proteins; and (e) a PE partially purified cell extract containing at least 1 naturally occurring proteins. Also claimed are: (a) an isolated or purified DNA encoding a protein as above, degenerate forms, variants or DNA hybridisable to the complement of the DNA; (b) a non-naturally occurring composition comprising (I) and at least 1 DNA polymerase; (c) an antibody binding (I); (d) a DNA construct comprising a sequence encoding PE protein P45 operably linked to an expression vector; (e) a P45 protein produced from a cell containing DNA construct as in (d); (f) a PE complex comprising P45; and (g) an antibody binding P45.

USE (I) improves the polymerisation activity of nucleic acid polymerases. (I) thus provides replication products of greater length and purity. Nucleic acid polymerase reactions can be enhanced (claimed) by mixing a NA template, at least 1 polymerase and (I). Kits are provided for replicating nucleic acids and comprise (I) and at least 1 polymerase (preferably recombinant). The kit can be used in site-directed mutagenesis, nucleic acid sequencing or amplification (preferably polymerase chain reaction, or reverse transcriptase-PCR).

ABSTRACTED-PUB-NO:

US 6333165B

EQUIVALENT-ABSTRACTS:

A new non-naturally occurring composition (I) comprises at least 1 components with nucleic acid polymerase enhancing (PE) activity selected from: (a) an isolated or purified natural PE protein from a bacterial, eukaryotic or archael source; (b) a wholly or partially synthetic protein having the same sequence as the natural protein, or an analogue with PE activity; (c) PE mixts. of at least 1 natural, or wholly or partially synthetic proteins; (d) a PE protein complex of at least 1 natural or synthetic proteins; and (e) a PE partially purified cell extract containing at least 1 naturally occurring proteins. Also claimed are: (a) an isolated or purified DNA encoding a protein as above, degenerate forms, variants or DNA hybridisable to the complement of the DNA; (b) a non-naturally occurring composition comprising (I) and at least 1 DNA polymerase; (c) an antibody binding (I); (d) a DNA construct comprising a

sequence encoding PE protein P45 operably linked to an expression vector; (e) a P45 protein produced from a cell containing DNA construct as in (d); (f) a PE complex comprising P45; and (g) an antibody binding P45.

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A new non-naturally occurring composition (I) comprises at least 1 components with nucleic acid polymerase enhancing (PE) activity selected from: (a) an isolated or purified natural PE protein from a bacterial, eukaryotic or archael source; (b) a wholly or partially synthetic protein having the same sequence as the natural protein, or an analogue with PE activity; (c) PE mixts. of at least 1 natural, or wholly or partially synthetic proteins; (d) a PE protein complex of at least 1 natural or synthetic proteins; and (e) a PE partially purified cell extract containing at least 1 naturally occurring proteins. Also claimed are: (a) an isolated or purified DNA encoding a protein as above, degenerate forms, variants or DNA hybridisable to the complement of the DNA; (b) a non-naturally occurring composition comprising (I) and at least 1 DNA polymerase; (c) an antibody binding (I); (d) a DNA construct comprising a sequence encoding PE protein P45 operably linked to an expression vector; (e) a P45 protein produced from a cell containing DNA construct as in (d); (f) a PE complex comprising P45; and (g) an antibody binding P45.

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US 6379553B

A new non-naturally occurring composition (I) comprises at least 1 components with nucleic acid polymerase enhancing (PE) activity selected from: (a) an isolated or purified natural PE protein from a bacterial, eukaryotic or archael source; (b) a wholly or partially synthetic protein having the same sequence as the natural protein, or an analogue with PE activity; (c) PE mixts. of at least 1 natural, or wholly or partially synthetic proteins; (d) a PE protein complex of at least 1 natural or synthetic proteins; and (e) a PE partially purified cell extract containing at least 1 naturally occurring proteins. Also claimed are: (a) an isolated or purified DNA encoding a protein as above, degenerate forms, variants or DNA hybridisable to the complement of the DNA; (b) a non-naturally occurring composition comprising (I) and at least 1 DNA polymerase; (c) an antibody binding (I); (d) a DNA construct comprising a sequence encoding PE protein P45 operably linked to an expression vector; (e) a P45 protein produced from a cell containing DNA construct as in (d); (f) a PE complex comprising P45; and (g) an antibody binding P45.

USE (I) improves the polymerisation activity of nucleic acid polymerases. (I) thus provides replication products of greater length and purity. Nucleic acid polymerase reactions can be enhanced (claimed) by mixing a NA template, at least 1 polymerase and (I). Kits are provided for replicating nucleic acids and comprise (I) and at least 1 polymerase (preferably recombinant). The kit can be used in site-directed mutagenesis, nucleic acid sequencing or amplification (preferably polymerase chain reaction, or reverse transcriptase-PCR).

WO 9842860A

CHOSEN-DRAWING: Dwg.0/44

TITLE-TERMS: POLYMERASE ENHANCE FACTOR PROTEIN EXTRACT COMPLEX IMPROVE POLYMERISE  
ACTIVE NUCLEIC ACID AMPLIFY SEQUENCE REPLICA

DERWENT-CLASS: B04 D16

CPI-CODES: B04-E02F; B04-E03F; B04-G01; B04-L04A; B04-N03; B11-C08E3; B11-C08E4;  
D05-H11; D05-H12A; D05-H12E; D05-H14; D05-H17C; D05-H18A; D05-H18B; D05-H19B;

CHEMICAL-CODES:

Chemical Indexing M1 \*01\*

Fragmentation Code

M423 M710 M903 N134 N135 Q233 V752 V753

SECONDARY-ACC-NO:

CPI Secondary Accession Numbers: C1998-162870

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
Image									

RMC	Draw Desc
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Term	Documents
P45.DWPI,USPT.	454
P45S	0
PROTEIN.DWPI,USPT.	184759
PROTEINS.DWPI,USPT.	116533
(3 AND (P45 ADJ PROTEIN)).USPT,DWPI.	4
(L3 AND P45 PROTEIN).USPT,DWPI.	4

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## WEST

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L10: Entry 6 of 7

File: USPT

May 9, 2000

DOCUMENT-IDENTIFIER: US 6060245 A

TITLE: Methods and adaptors for generating specific nucleic acid populations

Detailed Description Paragraph Right (258):

L cell and C5a adaptor-ligated cDNA were amplified to generate a sufficient amount of adaptor-ligated cDNA for denaturing and annealing the cDNA. This step is included as a convenient method to prepare additional adaptor-ligated cDNA and may be omitted by preparing a sufficient amount of adaptor-ligated cDNA as described in the previous step. The example herein uses a combination of cloned Pfu DNA polymerase and Polymerase Enhancing Factor (U.S. patent application Ser. No. 08/822,774, filed Mar. 21, 1997, "Polymerase Enhancing Factor `PEF` Extracts, PEF Proteins and Methods for Purifying and Identifying Same") to amplify the cDNA. Alternatively, other polymerases or polymerase mixtures are used. In the amplification reaction, it is critical that the both strands of the cDNA be complete and that they contain the adaptor sequences having the PCR priming sites and Eam 11041 recognition site. The ability of a polymerase or polymerase mixture and/or amplification conditions to create double-stranded cDNA was determined as follows.

Detailed Description Paragraph Right (260):

Specifically, 200 ng of the R and S adaptor primers were labeled as described in Eam 11041 Incubation with Single- and Double-Stranded DNA. The primers were then used to amplify the cDNA with R and S adaptors as follows: 100 ng adaptor-ligated cDNA is combined with 200 ng of each labeled primer, 1.times.cloned Pfu DNA polymerase reaction buffer, 2.5 units of cloned Pfu DNA polymerase and 7 ng Polymerase Enhancing Factor. The adaptor-ligated cDNA was amplified as follows: one cycle of 93.degree. C. for 3 minutes, 54.degree. C. for 3 minutes and 72.degree. C. for 2 minutes; 10 cycles of 93.degree. C. for 1 minute, 54.degree. C. for 1 minute and 72.degree. C. for 2 minutes; 2 cycles of 93.degree. C. for 1 minute, 54.degree. C. for 1 minute and 72.degree. C. for 2.25 minutes; 2 cycles of 93.degree. C. for 1 minute, 54.degree. C. for 1 minute and 72.degree. C. for 2.5 minutes; 2 cycles of 93.degree. C. for 1 minute, 54.degree. C. for 1 minute and 72.degree. C. for 2.75 minutes; and 72.degree. C. for 10 minutes. The amplification products were purified and concentrated with Centricon-100 Concentrators as described above. One tenth of the purified amplification products were incubated with 24 units of Eam 11041 at 37.degree. C. for 2 hours. The digestion products were analyzed by acrylamide gel electrophoresis and exposure to BIOMAX film for 3 to 4 hours to determine the size distribution of the labeled DNA. A similar reaction without Eam 11041 was prepared, incubated and analyzed in parallel. The method was also applied to R and S adaptor-ligated pSK-. R and S adaptors were ligated to pSK- using the same methods used in this example for cDNA.

Detailed Description Paragraph Right (264):

The use of additives which may enhance a desired result such as a change in priming specificity of the primer and template or a change in the polymerase activity and/or processivity of one or more polymerases in a primer extension reaction and the like are also contemplated. Exemplary suitable additives in primer extension reactions are Perfect Match.RTM. DNA polymerase enhancer (U.S. Pat. No. 5,449,603; Stratagene; La Jolla, Calif.), Polymerase Enhancing Factor (U.S. patent application Ser. No. 08/822,774, filed Mar. 21, 1997, "Polymerase Enhancing Factor `PEF` Extracts, PEF Proteins and Methods for Purifying and Identifying Same"), mutS (Wagner, R., et al., Nucleic Acids Res. 23:3944-3948, 1995 and Takamatsu, S., et al., Nucleic Acids Res. 24:640-647, 1996; Epicenter, Technologies, Madison, Wis.), betaine (Baskaran, N., et al., Genome Methods 6:633-638, 1996; U.S. Pat. No. 5,545,539; Sigman, St. Louis, Mo.),

dimethyl sulfoxide (DMSO; Hung, T., et al., Nucleic Acids Res., 18:4953, 1990; Sigma, St. Louis, Mo.), formamide (Sarkar, G., et al., Nucleic Acids Res. 18:7464, 1990; Stratagene, La Jolla, Calif.), tetramethylammonium chloride (TMAC; Chevet, E., et al., Nucleic Acids Res. 23:3343-3334, 1995; Sigma, St. Louis, Mo.), T-7 type single stranded DNA binding protein (U.S. Pat. No. 5,534,407), gene 32 protein of phage T4 (Schwarz, K., et al., Nucleic Acids Res., 18:1079, 1990) and the like.

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L10: Entry 6 of 7

File: USPT

May 9, 2000

DOCUMENT-IDENTIFIER: US 6060245 A

TITLE: Methods and adaptors for generating specific nucleic acid populations

Detailed Description Paragraph Right (258):

L cell and C5a adaptor-ligated cDNA were amplified to generate a sufficient amount of adaptor-ligated cDNA for denaturing and annealing the cDNA. This step is included as a convenient method to prepare additional adaptor-ligated cDNA and may be omitted by preparing a sufficient amount of adaptor-ligated cDNA as described in the previous step. The example herein uses a combination of cloned Pfu DNA polymerase and Polymerase Enhancing Factor (U.S. patent application Ser. No. 08/822,774, filed Mar. 21, 1997, "Polymerase Enhancing Factor `PEF` Extracts, PEF Proteins and Methods for Purifying and Identifying Same") to amplify the cDNA. Alternatively, other polymerases or polymerase mixtures are used. In the amplification reaction, it is critical that the both strands of the cDNA be complete and that they contain the adaptor sequences having the PCR priming sites and Eam 11041 recognition site. The ability of a polymerase or polymerase mixture and/or amplification conditions to create double-stranded cDNA was determined as follows.

Detailed Description Paragraph Right (260):

Specifically, 200 ng of the R and S adaptor primers were labeled as described in Eam 11041 Incubation with Single- and Double-Stranded DNA. The primers were then used to amplify the cDNA with R and S adaptors as follows: 100 ng adaptor-ligated cDNA is combined with 200 ng of each labeled primer, 1.times.cloned Pfu DNA polymerase reaction buffer, 2.5 units of cloned Pfu DNA polymerase and 7 ng Polymerase Enhancing Factor. The adaptor-ligated cDNA was amplified as follows: one cycle of 93.degree. C. for 3 minutes, 54.degree. C. for 3 minutes and 72.degree. C. for 2 minutes; 10 cycles of 93.degree. C. for 1 minute, 54.degree. C. for 1 minute and 72.degree. C. for 2 minutes; 2 cycles of 93.degree. C. for 1 minute, 54.degree. C. for 1 minute and 72.degree. C. for 2.25 minutes; 2 cycles of 93.degree. C. for 1 minute, 54.degree. C. for 1 minute and 72.degree. C. for 2.5 minutes; 2 cycles of 93.degree. C. for 1 minute, 54.degree. C. for 1 minute and 72.degree. C. for 2.75 minutes; and 72.degree. C. for 10 minutes. The amplification products were purified and concentrated with Centricon-100 Concentrators as described above. One tenth of the purified amplification products were incubated with 24 units of Eam 11041 at 37.degree. C. for 2 hours. The digestion products were analyzed by acrylamide gel electrophoresis and exposure to BIOMAX film for 3 to 4 hours to determine the size distribution of the labeled DNA. A similar reaction without Eam 11041 was prepared, incubated and analyzed in parallel. The method was also applied to R and S adaptor-ligated pSK-. R and S adaptors were ligated to pSK- using the same methods used in this example for cDNA.

Detailed Description Paragraph Right (264):

The use of additives which may enhance a desired result such as a change in priming specificity of the primer and template or a change in the polymerase activity and/or processivity of one or more polymerases in a primer extension reaction and the like are also contemplated. Exemplary suitable additives in primer extension reactions are Perfect Match.RTM. DNA polymerase enhancer (U.S. Pat. No. 5,449,603; Stratagene; La Jolla, Calif.), Polymerase Enhancing Factor (U.S. patent application Ser. No. 08/822,774, filed Mar. 21, 1997, "Polymerase Enhancing Factor `PEF` Extracts, PEF Proteins and Methods for Purifying and Identifying Same"), mutS (Wagner, R., et al., Nucleic Acids Res. 23:3944-3948, 1995 and Takamatsu, S., et al., Nucleic Acids Res. 24:640-647, 1996; Epicenter, Technologies, Madison, Wis.), betaine (Baskaran, N., et al., Genome Methods 6:633-638, 1996; U.S. Pat. No. 5,545,539; Sigman, St. Louis, Mo.),



dimethyl sulfoxide (DMSO; Hung, T., et al., Nucleic Acids Res., 18:4953, 1990; Sigma, St. Louis, Mo.), formamide (Sarkar, G., et al., Nucleic Acids Res. 18:7464, 1990; Stratagene, La Jolla, Calif.), tetramethylammonium chloride (TMAC; Chevet, E., et al., Nucleic Acids Res. 23:3343-3334, 1995; Sigma, St. Louis, Mo.), T-7 type single stranded DNA binding protein (U.S. Pat. No. 5,534,407), gene 32 protein of phage T4 (Schwarz, K., et al., Nucleic Acids Res., 18:1079, 1990) and the like.

**WEST**

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L4: Entry 2 of 41

File: USPT

Apr 2, 2002

DOCUMENT-IDENTIFIER: US 6365575 B1

TITLE: Gene delivery and expression in areas inaccessible to direct protein delivery

Detailed Description Paragraph Right (4):

Transfection with the AAT plasmid resulted in maximal levels of AAT in the medium of 300-800 ng/ml. FIG. 1 shows viral replication in the cell line studied as plaque forming units (PFU) in cells which received no treatment, cells exposed to RSV after transfection with AAT and cells exposed to exogenous AAT at a concentration of 30,000 ng/ml in the medium. The effects of the exogenous protein and transfection with the AAT gene were substantially different. Transfection with AAT markedly reduced RSV infectivity, but exogenous protein had little effect.

Detailed Description Paragraph Right (86):

Except for the cDNA for .alpha..sub.1 -antitrypsin and the 3'UTR of human growth hormone which were sequenced using the Sanger dideoxy method, the sequence is compiled using the current version of Genbank as source of the sequence information. The reading frame of the .alpha..sub.1 -antitrypsin gene is amplified using Vent DNA polymerase, 100 ng of target DNA, a programmable temperature cycler, and standard reaction conditions (denaturing at 93.5.degree., annealing at 56.degree. and extension at 75.degree.). Vent DNA polymerase is used because it has a 3' to 5' proofreading activity in addition to enhanced stability at high temperature and a highly specific and processive 5' to 3' DNA polymerase activity. After PCR amplification, the unique restriction sites were cleaved with the appropriate restriction enzymes (ClaI; SmaI), the amplified gene is separated from the small fragments released by the action of the restriction enzymes and from unincorporated primers and nucleotides by gel filtration through a S-400 spin column. The amplified genes which now had cloning sites on each end were ligated into PCMV4 which had been previously cleaved with the same restriction enzymes which were utilized to prepare the cloning sites on the amplified gene.